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Optimal process water supplementation and ratio between corn gluten meal and soy protein concentrate to secure good physical pellet quality and minimize energy use during extrusion processing of fish feed

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Abstract

The objective of this experiment was to find out which combination of water/steam addition and plant protein ingredient mix that gives best physical quality and process economy. The design was, however, restrained by a demand to reach a specified bulk density, above 420 g/l, set in order to meet requirements for high oil absorption capacity and fast sinking pellets. The moisture come from three sources: steam in the conditioner, water in the conditioner and water in the extruder. Total process water supplementation was 28%, 33%, and 39% for each of 4 combinations (0-300 g/kg) of corn gluten meal (CGM) and soy protein concentrate (SPC). However, water and steam addition was done by free combination of the three sources, to meet the bulk density criterion. This may have reduced the possibility to conclude concerning the main effects of water supplementation and optimal ratio between CGM and SPC.

Holmen durability analyzes showed that PDI value was in range from 99.2 to 99.7%. High pellet density in range from 720-784 g/l was reflected on sinking percentage values were almost all diets had 100% score. Sinking speed was in range from 8.2 to 13.2 m/s. Reduction in SME was found in diets where CGM content increases from 10 to 30%. The results obtained in this study indicate that CGM and SPC have comparable effects on physical pellet quality and energy use in extrusion, and thereby process economy.

Keywords: Extrusion, Soya protein concentrate, Corn gluten meal, Physical quality, Specific mechanical energy

List of abbreviations

g	Gram
kg	Kilogram
l	Liter
µm	Micrometer
m	Meter
mm	Millimeter
s	Second
h	Hour
N	Newton
Nm	Newton meter
kW	Kilowatt
RPM	Rotations per minute
Wh	Watt-hour
ºC	Celsius
mBars	Millibars
SE	Standard error

Contents

1 Introduction

Aquaculture is becoming the fastest growing food industry, driven by increasing human population growth and need for new and cheaper sources of protein as well as the world's growing demand for seafood. The annual production in 2012 was 90.43 Mt (million tons) of seafood products, with a proportion of 74% of fish (more than 66 million tons) and 26% of marine macroalgae and shells (FAO, 2012). This forces the feed industry to improve feed efficiency and to find solution for new, cheaper protein ingredients.

Recently, more plant proteins are available on the world market in form of by-products of bio-fuel production (Prieler, 2009). This creates new opportunities for fish feed industry in order to find the new protein sources.

1.1 Fish meal as a feed ingredient

Fish meal (FM) is made from by-products of slaughtered farmed fish or from wild marine fish catch which can't be sold or used for human consumption. In different regions fish meal is made from different species. FM is a sustainable source of protein but in limited quantities because the resources available are not sufficient to satisfy the projected increase in demand (FIN, 2008). Fishery quotas are determined by governments, in accordance with international conventions, in order to ensure sustainability in their production. These quotas probably will not increase in the future.

Traditional land animals have been fed with dried fish a long time. In 8th century in Norway was established first process of extracting oil from herring pressing them between stones and wooden boards (Windsor, 2001). FM is found in feed for almost all traditional land animals and pets (FAO, 2013a).

Nutrient composition and protein value are directly related to protein content, digestibility and amino acids (AA) composition. FM contains 60-75% crude protein, 6-9.5% crude fat, 8-12% ash

(FAO, 2013). FM is rich in lysine, methionine, cystine, valine and other essential amino acids. FM is a good source of vitamins thiamine (B1), riboflavin (B2), pyridoxine (B6), cobalamin (B12) and minerals such as Ca, P, Mg, K, (FAO, 2013a). Fat in phospholipids form is important component of FM especially rich in EPA and DHA omega-3 fatty acids which provide a good omega-6 and omega-3 ratio in feed. The lack of anti-nutritional factors is main advantage of FM compared with plant proteins.

Aksnes et al. (1998) determined apparent digestibility coefficient (ADC) in rainbow trout (*Oncorhynchus mykiss*) to be 97.3% for high quality FM, 94.1% for medium quality FM and 85.3% for fair average quality. In the same research ADC of protein were 89.3, 86.4, and 76.7%. This leads us to conclusion that the FM is a high quality protein source with high digestibility.

1.2 Fish meal price

In generally price reflects market conditions and supply– demand relation. According to FAO (2012) from 1985 until 2010, marine aquaculture growth was 5 million tons per year. At the same period fish meal production was reduced by 35%. The result of this disbalance has been raising price. According to International Monetary Fund the average price for fish meal in July 1985 was 254 $ per ton, at the same time in 2013 price was 2250 $ per ton, almost 900% increase in price in 28 years (IMF, 1987). FM price vary during the year, the lowest price is at the end and at the begginig of year and the bigest during the farming season on the north hemisfere, from March until October (Mittaine 2014). At this moment one ton of fish meal cost 2000 $ per ton. While price for one ton of soybean meal (46% protein) is 327,30 $, for corn gluten meal (65% protein) 725$, for wheat is aprox. 190 $ and corn 160 $ per ton (Grains, 2015). The reason for high price lies in aquaculture development in and Europe, competition with feed industries for land animals. It might be possible that the fish meal price will be stable in the close future because of new fishmeal manufacturer from Africa, South America and Asia and because of incorporation of new plant ingredients in fish feed.

1.2.1 Alternatives to fish meal

Scientists around the world are looking for new, renewable, high quality and inexpensive sources of protein, which may partially or completely replace fish meal in diet for farmed fish and crustacean species. Vegetable proteins such as soy protein concentrate (SPC), pea protein, lupin protein, by-products from ethanol and biodiesel industry, corn and wheat gluten meal, single cell organisms such as algae protein, bacterial meal, yeasts, have lately been imposed as a possible source of protein that could replace fish meal. By simple comparison, price for medium quality FM protein is almost three times as high as protein from corn gluten meal (CGM) and the crude protein content is almost the same. Regarding to this facts CGM might be potentially good substitute for FM in the future.

1.3 Soy and soy protein concentrate

Soy is one of the most commonly used protein-rich plants in a feed industry. It is found in several different forms such as full-fat soybean, soybean meal, untoasted defatted soybean, white flakes and soybean cake (Fallahi et al., 2012). All forms soy derivates are used in the feed industry due to the high protein content. Soy protein is challenging due to the lack of lysine and methionine and presence of antinutritional factors (ANF) and non starch polysaccharides (NSP) (Fallahi et al., 2012). SPC is modified, thermal treated form of white flakes where ANF and NSP are reduced and concentration of essential amino acids greater than in FM (Fallahi et al., 2012). High production cost force us to find new, inexpensive source of protein which can partially replace SPC.

1.4 Corn gluten meal

CGM is a by-product from corn starch production. The production of corn starch is increasing due to the use in processing of ethanol for fuel (Hoffman et al., 2010). CGM crude protein content is approximately 65% in dry mater (RFA, 2008). CGM is used in diet for almost all land animals including fish and pets. In modern feed industry CGM is used as an energy and protein sources well as a pigment for some fish species. The corn gluten is commonly used for pet food because of the high protein digestibility (RFA, 2008). CGM can be found in wet or dried form. Due to transport and shelf life of the material, the dried form is most used.

1.4.1 Price for corn gluten meal

Corn gluten meal compared to fish meal is an inexpensive protein source with a current price at 711 $ per ton (Grains, 2015). In the last decade, price was more or less stable, but during this decade the price level has doubled. Crude protein content in FM is approximately 5% more than in CGM, however the price of FM compared to CGM is almost 250% higher. This suggests that the corn gluten might be potentially good substitute.

1.4.2 Corn gluten meal as a feed ingredient

From the nutritional point of view, fish meal and corn gluten meal have certain difference in amino acid composition. Fish meal is considered to be high in lysine, arginine, threonine and tryptophan. On other side CGM amino acid profile is considered as a poor in lysine (1.7% compared to7.5% for FM) and tryptophan (0.5% compared to 1.1% for FM) (Leeson et al., 2005). CGM is considered to be rich in glutaminic acid and leucine. Serine, methionine and alanine are on the similar level and other amino acids have no significantly different (Yu et al., 2013; Jensen, 1990). Ribeiro et al. (2012) compared ADC and TDC of protein and amino acids for Nile tilapia (*Oreochromis niloticus*) in the growth phase. Six diets with a different protein source showed that soybean meal (SBM) and CGM had the highest ADC of protein and amino acids 86.1% and 85.19%, while ADC were 73.74% for wheat bran and 76.74% for FM respectively.

In some fish species flesh flavor, skin and flesh pigmentation, may affect price, attract or detract consumers. According to the Adelizi et al. (1998), corn gluten meal did not affect flesh flavor negatively in rainbow trout (*Oncorhynchus mykiss*). Rainbow trout fed with increasing levels of CGM showed significant linear reduction in the concentration of astaxanthin, all-trans lutein and all-trans astaxanthin pigments (Seaz et al. 2014). In order to avoid unwanted pigmentation Seaz (2014) recommends that the level of CGM in a feed should not exceed 9%.

1.5 Fish feed

Well manufactured feed is one of the most important production factors in modern aquaculture. It has to integrate all fish nutritional demands for essential amino acids, lipids, vitamins, macro and micro minerals in to each single pellet, and to provide good physical quality and competitive price.

In early stage fish larva might be fed with live feed or feed in granular form. Different types of feed are used for different fish species in different regions. For some species, such as herrings, semi-moist feed is used (FAO, 2013b). The dry feed, with less than 10% moisture, is used for salmonids.

Several different technologies are used in fish feed industry depending on the type of fish. Extrusion technology is main technology used in fish feed production for the last 30years. Early beginning of the extrusion technology was in 1940's for processing the cereal grain with a single screw extruder (Ferouz et al., 2011). Twin screw extrusion was established in 1970's with wider range of products suitable for processing (Harper, 1989) and it represents the major technology for production of the aquatic feeds today. The extrusion process creates wide range of pellets with different dimensions and shapes, adjusted for different species in a different life stage and for different fish size. Different extrusion settings such as pressure, temperature, water content, screw configuration and screw speed allows pellets density control, improving durability and physical quality of pellets and efficient production (Kiang, 1998; Brent,1989). Extrusion leads to the better hygiene in fish feed and at the same time less problems with diseases in aquaculture (Kiang, 1989). Appropriate grinding, mixing and conditioning of ingredients prior to extrusion, in order to make a homogenous mash, are necessary.
In some developing countries in Asia and Africa pelleting is still used in fish feed production.

1.6 Factors affecting feed quality

Feed quality is determined by physical, nutritional, hygienic and sensorial quality (Sørensen, 2003). Among others the accent in this thesis will be on physical quality of pellets which can be defined as ability of pellets to withstand handling without creating the dust (Sørensen, 2003). In order to reduce the dust and to maximize feed intake, high technical quality is required.

1.6.1 Chemical factors affecting feed quality

1.6.2 Carbohydrates

The common form of polysaccharides found in a feed is starch (Oliveira, 1990). The starch molecule is composed of amylose and amylopectin molecules. The starch granules are water insoluble, but during the grinding process starch is broken, properties are changed and water is more easily absorbed. With the presence of water and heat starch granules start swelling which increase viscosity and allows gelatinization. Therefore steam addition in the pre-conditioning process is crucial in order to achieve high or complete gelatinization. In a salmonids fish feed starch has limited usage. In generally salmonids have low ability to digest starch (Lovell, 2002) and the starch is mainly used to improve binding properties and expansion of pellets (Oliveira, 1990). Starch gelatinization is highly affected by available water amount (Storebakken et al.,2015a). Digestion of lipids in monogastric animals may be affected by indigestible carbohydrates (Storebakken et al.,2015a). Expansion volume is affected by starch composition or amylose-amylopectin ratio as well as degree of gelatinization affects the expansion ratio (Chinnaswamy& Hanna, 1988).

1.6.3 Lipids

In a feed for salmonids different types of oil are added due to high energy requirements. Lipids are the main energy source in salmonids, due to the metabolic limitations in tolerating and utilizing high quantities of starch. Storebakken et al.(2015a) have found connection between apparent digestibility (AD) of starch and lipids. Every 10% increase in AD of starch, increases AD of lipids by 0.6% in a rainbow trout (*Oncorhynchus mykiss*) diets with hydrolyzed wheat gluten (HWG). Lipids are found in almost all protein-rich main ingredients used in feed. High lipid content before extrusion gives hydrophobic features to the feed. Lipids can negatively affect the starch properties and gelatinization during the extrusion process due to their lubricating effect (Riaz 2000), and may also result oxidation of the feed due to degradation of lipids initiated by the high temperature and high moisture content applied during extrusion (Camire et al., 1990). Because of these features, most of the oil addition is mainly performed with vacuum coaters, after extrusion and drying.

1.6.4 Protein

In salmonids feed proteins are mainly used as amino acid source. Proteins from the different plants in combination with starch may have different effect on expansion of pellets. Faubion et al., (1982) have reported that combination of pure starch soy protein isolate increase expansion of extrudate. Water absorption index and expansion may be affected with increased protein content (Matthey & Hanna, 1997). Wood (1987) reported that partial degradation of protein structure has positive effect on pellet durability.

1.6.5 Water, steam and pressure and its influence on feed pellet quality

Water is one of the most important factors affecting the pellet expansion. Passing the die, reduction from high to atmospheric pressure is causing evaporation of water and pellet expansion (Sørensen, 2003). Rokey (1994) has recommended that optimal level of water during the extrusion process should be between 25-30%. If the water content is to low starch granules will not swell and starch gelatinization will be affected negatively. Water in a higher amounts will reduce viscosity, friction between particles, screw and extruder barrel. Less friction means lower temperature and less expansion rate and heat should be added. Pellet durability and hardness are improved with an increasing amount of steam in pre-conditioner (Thomas et.al., 1997). Oliveira (1990) has reported that steam addition reduces costs. Physical pellet quality was improved by increasing steam pressure (Brisset, 1992). The rate of expansion might be affected negatively if the steam pressure is higher than 7 MPa (Gomez & Aguilera, 1983).

1.7 Extrusion technology processing steps and factors affecting feed quality

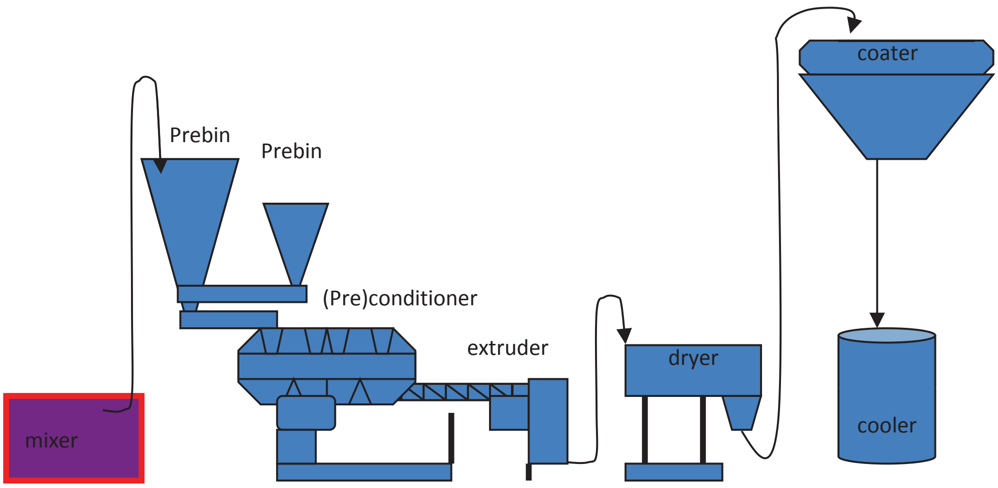

Figure 1

Extrusion processing steps (Kraugerud, 2013)

1.7.1 Grinding and its effect on feed quality

Milling is first processing step. Particle size reduction is required prior to mixing in order to improve mash homogeneity and to avoid segregation. Increased surface area allows that water and heat can easily gelatinize starches and denature the proteins (Sørensen, 2003). Particle size reduction improves physical and nutritional quality of feed (Sørensen, 2003). Particle size reduction significantly improves feed durability as well as water stability (Oblado et al.,1998). Bühler recommends that particle size should be smaller than 70% of the die diameter (Bühler, 2015). Larger particles affect expansion negatively. Hammer mill and roller mill are most commonly used machines for particle size reduction. Particle size is determined by the screen size on the hammer mill or by the gap between rollers on the roller mill.

1.7.2 Dosing and mixing and their effect on feed quality

Fish feed may contain nearly or more than 20 different macro and micro ingredients. Optimal composition is essential for good feed utilization. Appropriate dosing has to ensure exact amount of each ingredient. After dosing, ingredients are transported to mixer. Mixing is required prior extrusion in order to mix different macro and micro ingredients into a homogenous mixed mash. Mixing time depends on diet composition. Different liquids can be added into mixer. The most commonly used machine is twin shaft pedal mixer. Mixed mash is transported to the feeders and supply bins mounted above extruder.

1.7.3 Pre-conditioner and its influence on the power consumption and feed quality

Pre-conditioner is a chamber before the extruder. Moisture and heat are added under constant mixing. Pre conditioner might be pressurized or atmospheric. The main purpose is to moisturize and heat mash and start cooking of the starch and denaturation of proteins prior to the extrusion. Increased extruder capacity and reduced mechanical power consumption are main advantages of pre-conditioner use (Strahm et al., 2000). The retention time is defined with level of fat so diets with high fat content have retention time up to 290 seconds (Strahm et al., 2000). Average retention time is 120-180 seconds (Strahm et al., 2000). The most commonly used pre-conditioner in fish feed industry is double shaft counter rotating conditioner. After pre-conditioning feed mash enters the extruder barrel.

1.7.4 Extruder and its influence on the power consumption and feed quality

Extruder can be classified in to three groups: single screw, twin screw co-rotating and twin screw counter-rotating. Twin screw co-rotating extruders are most commonly used in the fish feed industry. The main advantages of co-rotating extruder are good self cleaning, high pumping efficiency and uniformity of processing (Riaz, 2000). The extruder barrel, shafts placed inside, heads and shear locks are main extruder components. Different screw elements can be placed on shafts such as mixing, kneading and shearing. Normally, screw configuration is combination of conveying and mixing elements. The conveying elements generate the pressure necessary for the material flow through the die (Sørensen, 2003). The screw is usually divided into three, feed, kneading and final cooking zone (Sørensen, 2003). Feed zone is transport zone in which material is preheated to a melting temperature. Feeding zone is followed by kneading zone where melting and plasticization occurs (Sørensen, 2003). Screw force material forward from kneading zone to final cooking zone where pressure increase up to the level needed to push material through the die.

Specific mechanical energy (SME) is commonly used parameter in feed production. SME is considered as a total sum of mechanical energy used to produce one kilo of extrudate (Fayose et al., 2012). SME indicate the energy consumption and relative extrusion cost (Fayose et al., 2012). Bordoloi & Ganguly (2014) reported that the SME decreased by the increased moisture content during the extrusion of maize grits.

Expansion of pellets is caused by pressure, heat and moisture. Heat might be formed by mechanical energy due to friction between raw materials, extruder barrel wall and screws as well as due to moisture addition in the form of steam dispersed around the particle surface (Sørensen, 2003).

The die is placed at the end of extruder barrel. The main purpose is to create restriction to product flow and to build required pressure, shear and shape of pellets (Sørensen, 2003). The pellet shape is determined by the extruder die design and the die thickness.

1.7.5 Drying, cooling and their impact on physical quality

In order to avoid growth of bacteria and moulds, feed has to be dried to the moisture content below 10% (Sørensen, 2003). Hot air around the pellets force water to evaporate, decreasing the pellet bulk density, pellet size and packing characteristics as well as increasing porosity and solid type (Bühler, 2014). Drying can improve rigidity and crush strength of pellets as well as oil absorption and shelf life (Bühler, 2014).The feed after drying may hold temperature up to 60˚C. Before bagging feed needs to be cooled on temperature which should not exceed 10˚C of the ambient temperature in order to avoid condensation of water after bagging (Sørensen, 2003).

1.7.6 Vacuum coating

Vacuum coating is a post extrusion process. The vacuum coater creates the vacuum and disperses the oil on the pellets placed in its coater chamber. The oil is pushed inside of pellets when the vacuum is released. Throughout the entire process, mixing is constantly present. Highly porous pellets can absorb oil easier (Sørensen, 2003). The pellet porosity is considered as the percentage of pores area compared to the total area (Draganović et al., 2013). However, the porosity and its geometrical status in the pellets can have great importance.

1.8 Objectives of MSc thesis

The objectives of MSc thesis was to identify which type of water addition and ingredient mix give best physical quality and process economy.

2 Materials and Methods

2.1 Experimental design

The experiment was performed in order to reach a specified bulk density, above 420 g/l. The bulk density was set in order to meet requirements for high oil absorption capacity and fast sinking pellets. The main task was to find out which combination of water/steam addition and ingredient mix give best physical quality and process economy. Place and magnitude of steam/water was adjusted within each of 3 total moisture addition levels (28, 33 and 39%). This design was set and could not be influences for the research going into this MSc thesis.

Table 1

Experimental feed composition, ratio SPC, CGM and total water amount in different trials

DietName	SPC30-CGM0			SPC20-CGM 10			SPC10-CGM 20			SPC0-CGM 30		
Wheat	7.0			7.0			7.0			7.0		
Sunflower meal	3.0			3.0			3.0			3.0		
Fishmeal	15.0			15.0			15.0			15.0		
Fababean, dehulled	7.0			7.0			7.0			7.0		
SPC	30			20			10			0		
Corn gluten meal	0			10			20			30		
Water change	0.2			0.2			0.2			0.2		
Rapeseed oil	18.0			18.0			18.0			18.0		
Fishoil	16.5			16.5			16.5			16.5		
Mineral&Vitamin mix	3.4			3.4			3.4			3.4		
Total	100.0			100.0			100.0			100.0		
Trial	1	2	3	4	5	6	7	8	9	10	11	12
Total water %	39	33	28	39	33	28	39	33	28	39	33	28

2.2 Diets

The experimental diets were produced at Centre for Feed Technology (FôrTek), at the Norwegian University of Life Sciences. The experiment was carried out in order to find optimal process water supplementation from three sources: steam in the conditioner, water in the conditioner and water in the extruder. This was applied manually, beyond my control, in different levels for each diet in order to reach specified bulk density (Table 3). Variable feed ingredients were SPC and CGM, where SPC was gradually replaced by 0, 10, 20 and 30 % of CGM (Table 1).

The diets were ground and mixed at Skretting ARC Technology Plant (Stavanger, Norway). The dry ingredients were pre-mixed in a vertical mixer (custom designed; Skretting ARC, Stavanger, Norway) before grinding. Grinding of all diets was carried out with Dinnissen 30kW hammer mill (Dinnissen, Sevenum, The Netherlands) through 0.75 mm screen. The diets were mixed again in a Dinnissen horizontal ribbon mixer (500LTR, Sevenum, The Netherlands) for 7 minutes. After mixing, raw materials were packed in 500kg plastic bags and transported to "FôrTek" for extrusion processing.

2.3 Screw configuration, die design and knife settings

The screw configuration was designed in order to control mechanical energy and cooking temperature. Screw configuration was integrating: three conveying zones and two kneading zones converted in to cooking zones with 20 left (20L) elements needed to create backflow (Figure 2). Number in the element name represents length. Letters R and L are representing flow direction, forward (R) and backflow (L). Poligon right block element provides kneading, mixing and shearing of mixed mash.

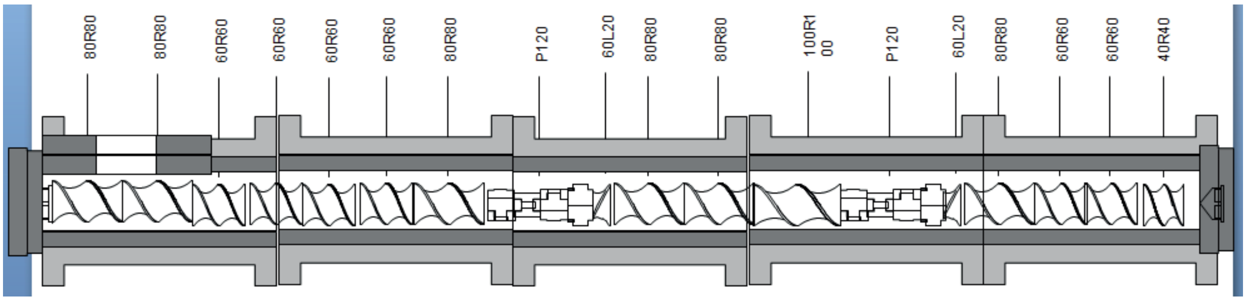

Figure 2

Screw configuration

The die plate contained one single die with a diameter of 6 mm. Pellet diameter was determined with screw speed and die diameter. Pellet length was regulated with knife speed and the number of knives (Table 2)

Table 2

Screw speed, knife speed and number of knifes for different diets

Diet		1	2	3	4	5	6	7	8	9	10	11	12
Screw speed	RPM	280	362	450	370	320	520	350	370	510	250	275	300
Knife speed	RPM	1300	1350	1700	1350	2050	1350	1500	1200	1400	2300	2300	2300
Number of knives		6	6	6	6	6	6	6	6	6	6	6	6

2.4 Extrusion processing

At the beginning of process, mixed mash passed through a Bühler (BTCT) (Bühler, Uzwil, Switzerland) pre-conditioner where water and steam are added, in a different amount for all diets. All diets were extruded in a twin-screw co-rotating extruder type of Bühler BCTG 62, with 5 barrel sections, driven by a 45kW electrical motor. Maximum production capacity of the extruder is 800 kg/h of fish feed. Capacity was regulated by feeder screw speed. Process parameters are shown in Table 3.

Table 3

Process parameters

Process parameters													
Diet		1	2	3	4	5	6	7	8	9	10	11	12
Temp. in the pre-conditioner	ºC	-	-	-	97.0	87.0	97.0	97.0	87.0	93.0	95.0	97.0	91.0
Extruding													
Water in conditioner	%	19.0	0.0	0.0	11.0	11.6	6.0	20.0	14.0	13.0	22.0	11.0	16.0
Steam in conditioner	%	10.0	8.0	8.0	9.0	13.5	9.0	12.0	9.0	8.0	11.0	18.0	9.0
Water in the Extruder	%	10.0	25.0	20.0	19.0	8.0	13.0	7.0	10.0	7.0	6.0	4.0	3.0
Total amount of water	%	39.0	33.0	28.0	39.0	33.1	28.0	39.0	33.0	28.0	39.0	33.0	28.0
Temp. in the extruder													
Section 1	ºC	81.0	84.2	84.9	83.2	80.4	78.7	88.6	88.6	78.7	94.0	95.7	90.7
Section 2	ºC	105.0	103.1	102.1	95.0	99.2	100.5	102.0	102.0	100.7	106.2	107.7	106.8
Section 3	ºC	113.0	109.5	122.3	106.9	110.0	123.1	112.1	112.1	119.0	110.2	113.7	111.9
Section 4	ºC	125.0	123.8	131.8	122.6	125.0	143.1	122.0	122.0	136.0	116.3	120.4	121.8
Section 5	ºC	124.3	116.4	112.5	122.4	126.8	134.9	119.7	119.2	129.5	114.9	118.2	121.3
Capacity	kg/h	200	200	200	268	300	280	270	270	270	200	200	200
Density	g/liter	425.0	420.0	420.0	421.0	420.0	420.0	420.0	420.0	420.0	425.0	422.0	420.0
Pressure	bar	17.0	17.8	19.5	15.0	19.0	12.0	11.0	11.0	13.8	10.5	10.7	13.4
Die Temp./Pressure	ºC	111.0	112.0	116.0	118.0	109.0	105.0	103.0	106.0	122.0	103.0	106.0	110.0
SME	Wh/kg	49.0	64.1	32.3	42.0	64.0	69.0	36.3	42.3	56.0	27.1	34.1	40.3
Torque	Nm	100.0	98.0	41.9	410.0	422.0	432.0	327.0	389.0	347.0	268.0	291.0	306.0

2.5 Post extrusion processes

2.5.1 Drying, cooling and moisture content

Drying process was done in order to reduce amount of unbound water in the product and to ensure long shelf life of product. Drying and cooling process were performed manually, with 3 manual, 50kg, batch dryers/coolers made by "FôrTek". The fan was a W2E 300-DA 01 W-160 (EBM-Papst, Mulfingen, Gemany) with capacity 2550 m^3/h. The heater was "Viking" (Viking, Denmark), power 10 kW with max air temperature 60˚C. Drying was performed at temperature 60˚C, 90 minutes for diets with 39% of added water, 75 minutes for diets with 33% of added water and 60 minutes for diets with 28% of added water. Cooling was performed in the same dryers/coolers by switching off heater, at room temperature 20˚C, 5 minutes for each diet. Moisture content (MC) for all trials were assessed by standard procedures EU 71/393.

2.5.2 Vacuum coating

Coating experiment was conducted with two different mixer-vacuum coaters. Forberg 0.37kW, F-6, twin-shaft paddle mixer-vacuum-coater was used in the first experiment, by creating a sub-atmospheric pressure of 200 mBars. Manual, 0.5l locally made lab drum-mixer-coater (NMBU-FôrTek, Ås, Norway) was used in the second experiment, six months after the production. The pellets were coated with 34.5% oil mixture made of 50% rapeseed oil and 50% of fish oil. The experiment had two replicas.

2.6 Physical quality analyzes

2.6.1 Particle size distribution analyses

According to the Thomas et al., (1996) particle size distribution affects fragmentation, abrasion and bulk density. Particle size analyzes were performed for mixed mash by using a Mastersizer 2000 laser-diffraction-analyzer (Malvern Instruments Ltd, Malvern, United Kingdom) which can measure particles >0.02 µm - 2000 µm. The test was carried out in duplicate measurements for each feed.

2.6.2 Flow rate analyses

Flow rate is a simple method developed by “Nofima”- Norway, in order to determine internal friction or flowing property of materials. Fifty grams of material is weighed and inserted into the 2.36 mm sieve. The material was scraped on the sieve in order to fill the cylinder under the sieve. Material which turn out the cylinder is returned back into the sieve until material cone is formed one the cylinder. The test was done when the material cannot build up the cone. The cone height value in cm represents flow number. The experiment was done for CGM and SPC diets with two replicate treatments per diet.

2.6.3 Pellet density analyses

Pellet density represents mass per volume. Depending on pellet density, different feed has different behavior in water such as fast-sinking, slow-sinking and floating pellets (Munz, 2004). As a pellet density increase, sinking speed is higher and opposite. After the coating, 1 l volume measuring cylinder was filled in to the top with the pellets in order to measure weight per volume. The test had three replicas for each diet.

2.6.4 Pellet durability analyses

Pellet durability index (PDI) represents amount of dust created during the transport, shearing and abrasion actions (Thomas et al., 1996). Lignotech Holmen portable tester (Borregaard Lignotech, Sarpsborg, Norway) simulates pellet shearing during the pneumatic handling (Thomas et al., 1996). Doris tester (Aquasmart ASA, Bryne, Norway) simulates stress, which occur during the pneumatic feeding systems. Durability test was done for all trials with Doris and Holmen testers. Approximately 100g of sieved sample was transferred in to Holmen portable tester. Retention time in Holmen tester was 120 seconds. Each test was carried out with three replicates.

2.6.5 Hardness test

Pellet hardness is static pressure force which affects pellets on the bottom by weight from the top (Tomas et al. 1996). Pellet breakage may occur on the bottom of bag or silo, during the storage (Tomas et al. 1996). Intestinal absorption of nitrogenous components is affected by hardness (Čuperlovic et al., 1973). Analyzes were done with Texture Analyzer (Tinius Olsen, Horsham, USA) by measuring diameter, the force needed to crush a pellet and the braking distance. The test had 30 replicas for each diet.

2.6.6 Sinking speed and sinking percentage analyses

Sinking speed was measured by sinking speed procedure, developed by Aquaculture Protein Centre, UMB. The pellets were dumped in to the measuring cylinder filled with water in order to analyze how many seconds pellet needs to sink one meter. Water salinity was 2.5%. The test had 30 replicas for each diet.

Sinking percentage was measured by number of sinking pellets after 15 seconds. Hundred pellets were randomly collected for each treatment. The pellets were dumped in to the measuring cylinder filled with water in order to analyze how many pellet will sink. Water salinity was 2.5%. The test had 3 replicas for each diet.

2.6.7 Fat leakage analyses

Fat leakage shows the maximum amount of oil which pellets can absorb and keep inside. The test was done after coating by putting pellets in to the bucket with the paper on the bottom and the 1000 g of sample was added. The bucket was covered with panel to protect sample from the dust. Room temperature was 40 ˚C. The next day pellets were weighed. The test had two replicates.

2.7 Data presentation and analysis

Curves, diagrams and correlation analyses were prepared with Microsoft excel 2007. Particle size distribution, fat leakage, maximal fat absorption, SME and applied energy are presented as mean values. Flow rate, pellet density, pellet durability, pellet hardness, sinking percentage and sinking speed analyzes are presented as mean values with standard errors of means.

3 Results

3.1 Particle size distribution

In order to identify particle size distribution of mixed mash, particle size analyzes were performed (Figure 3). In the CGM30 diet difference was observed compared to the other diets where particle size distribution analyzes showed that diet with 30 and 20% of CGM had higher volume of particles in range from 100 up to 1000 micrometers (Figure 3).

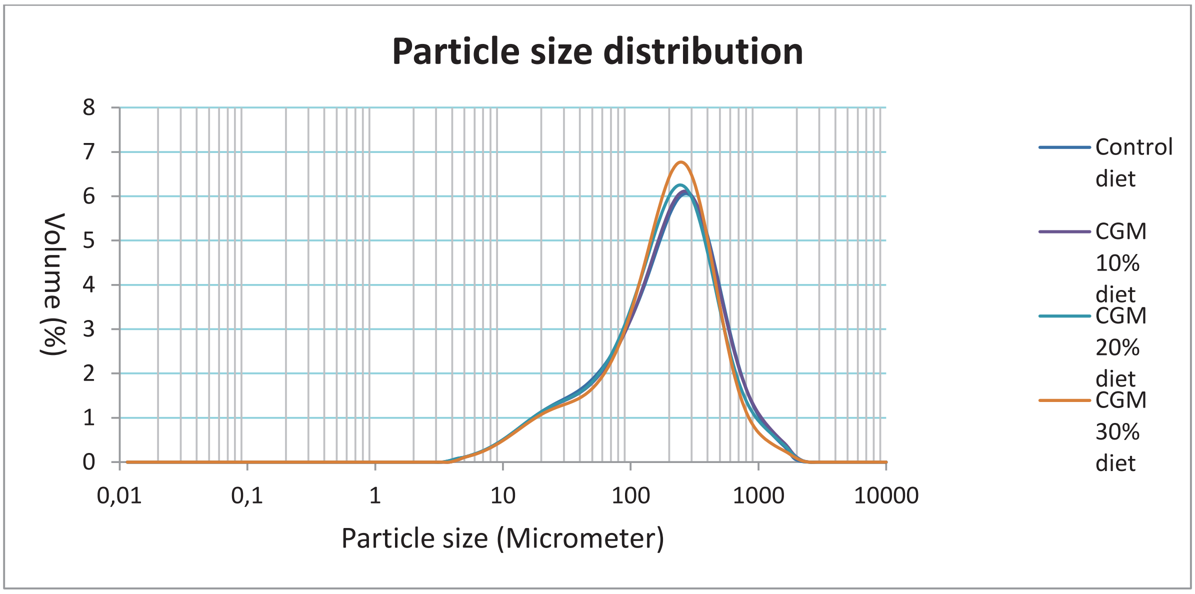

Figure 3

Particle size distribution (Volume %) mixed mash is presented for different diets. Results are presented as mean of two replicates.

3.2 Flow rate

In order to identify flow number, flow rate analyzes of CGM and SPC were performed (Figure 4). Reduction of flow rate was observed for CGM compared with SPC.

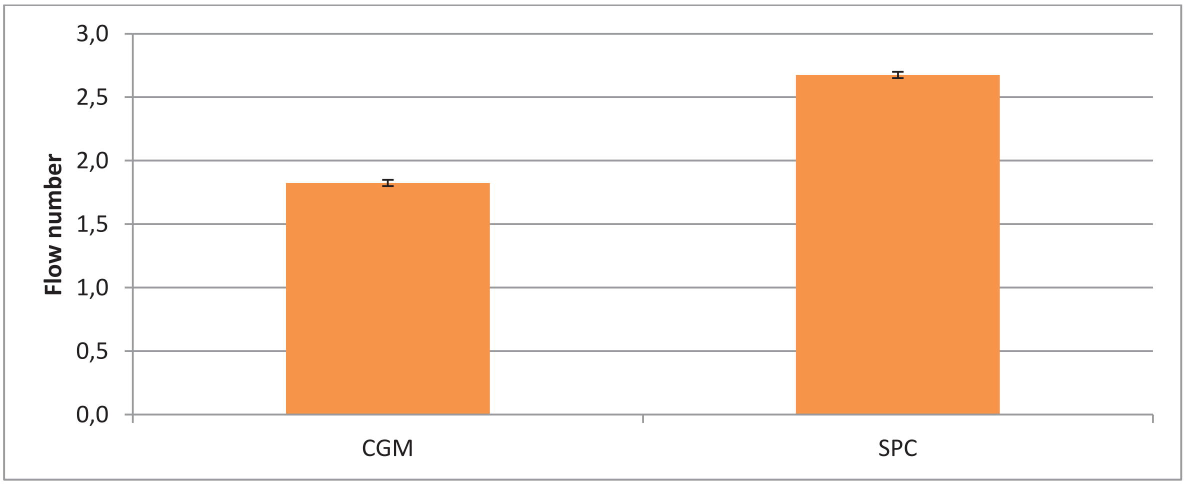

Figure 4

Flow rate analyzes. Flow number is presented for CGM and SPC. Results are presented as meanof two replicates ±SE.

3.3 Pellet density

In order to determinate density of coated pellets treated with different levels of water content and SPC and CGM, pellet density test was performed (Figure 5). Differences were observed on SPC30-CGM0 treated with 39% of moisture and SPC0-CGM30 treated with28% of moisture, compared to the other diets. In addition, increased pellet density was observed between different diets where level of CGM in the diet increases.

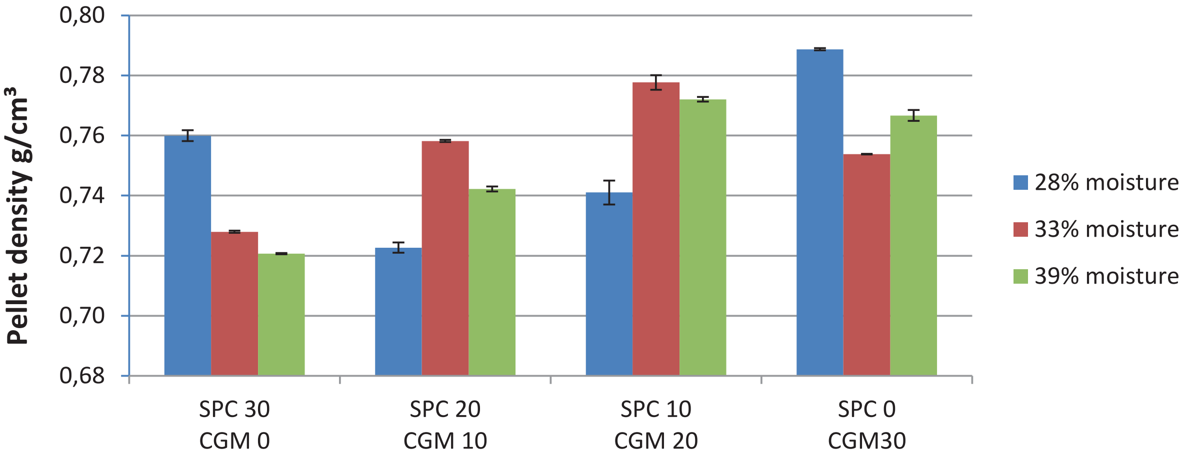

Figure 5

Pellet density analyzes. Pellet density is presented for different treatments. Results are presented as mean of three replicates ±SE.

3.4 Pellet durability

In order to identify pellet durability treated with different levels of water content SPC and CGM, Doris durability tests were performed after production and after 6 months of storage (Figures 6 and 7). The both Doris durability tests show similar results. In dietSPC30-CGM0 treated with 28% of moisture and SPC0-CGM30 treated with 28% of moisture, differences were observed compared to rest of the treatments. Reductions of durability were observed in diets with decreasing moisture levels with exception in a diet SPC10-CGM20 treated with 28% of moisture (Figures 6 and 7).

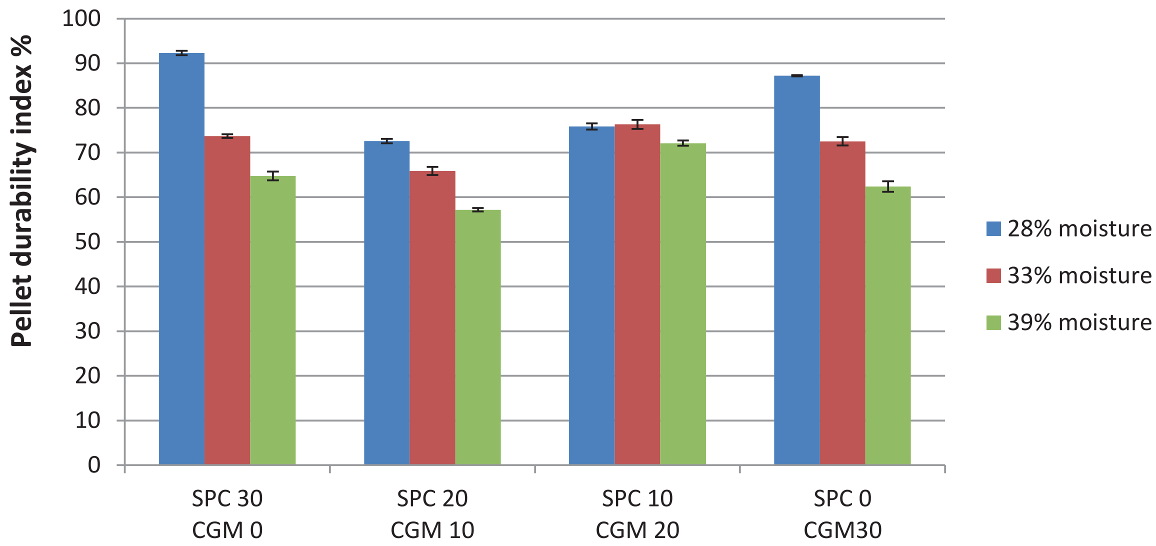

Figure 6

Doris feed test – done after production. Durability (%) of pellets is presented for different treatments. Results are presented as mean of three replicates ±SE.

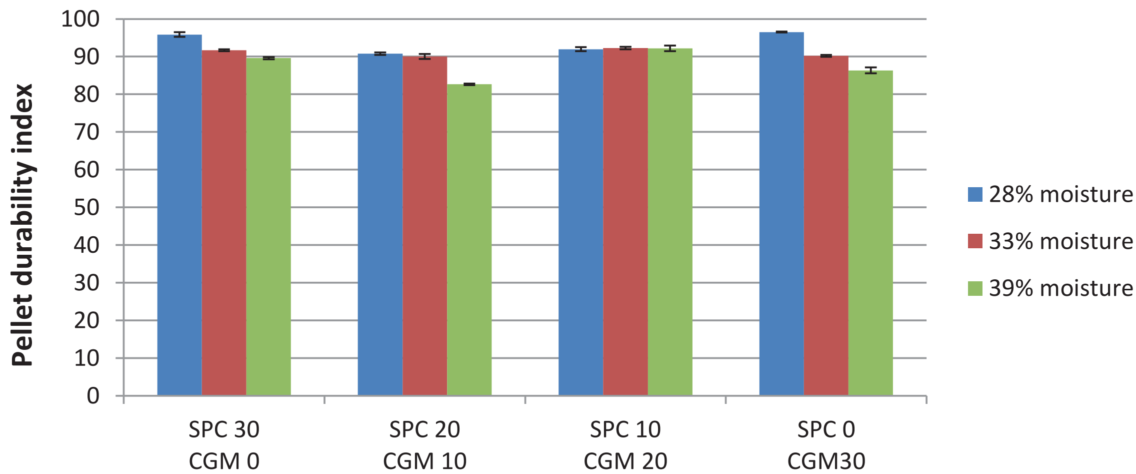

Figure 7

Doris feed test – done after 6 months of storage. Durability (%) of pellets is presented for different treatments. Results are presented as mean of three replicates ±SE

Holmen test was performed after production in order to identify pellet durability treated with different levels of water content, SPC and CGM (Figure 8). In diets SPC20-CGM10 treated with 33 and 39% of moisture, differences are observed compared to the rest of the results. In addition reduction in pellet durability was observed in other treatments.

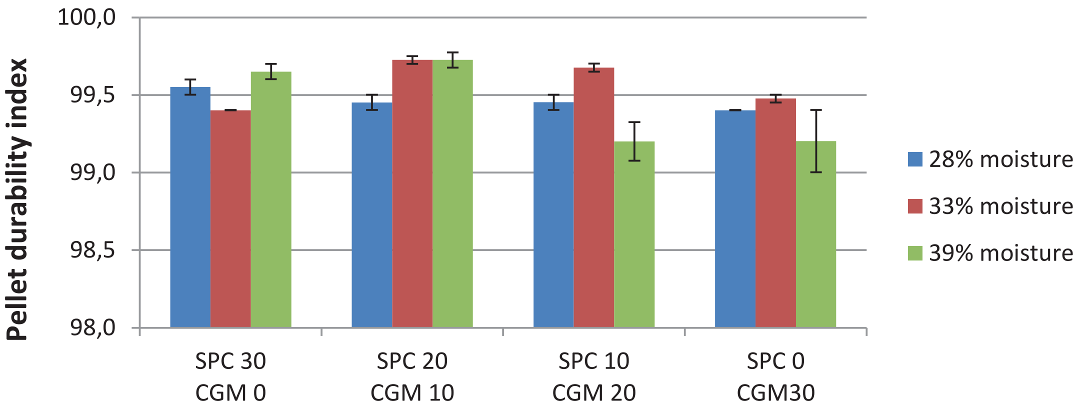

Figure 8

Holmen durability test – done after production. Durability(%) of pellets is presented for different treatments. Results are presented as mean of three replicates ±SE

3.5 Hardness analyzes

Hardness analyzes were performed in order to identify and compare static pressure force which pellets, treated with different levels of water content, SPC and CGM, can resist. Hardness tests were performed with texture analyzer after 6 months of storage (Figure 9). In diets SPC30-CGM0 treated with moisture 28%, SPC10-CGM20 treated with moisture 33% and SPC0-CGM30 treated with 28% moisture, differences were observed compared to rest of the treatments. Reduction of hardness in other diets was observed.

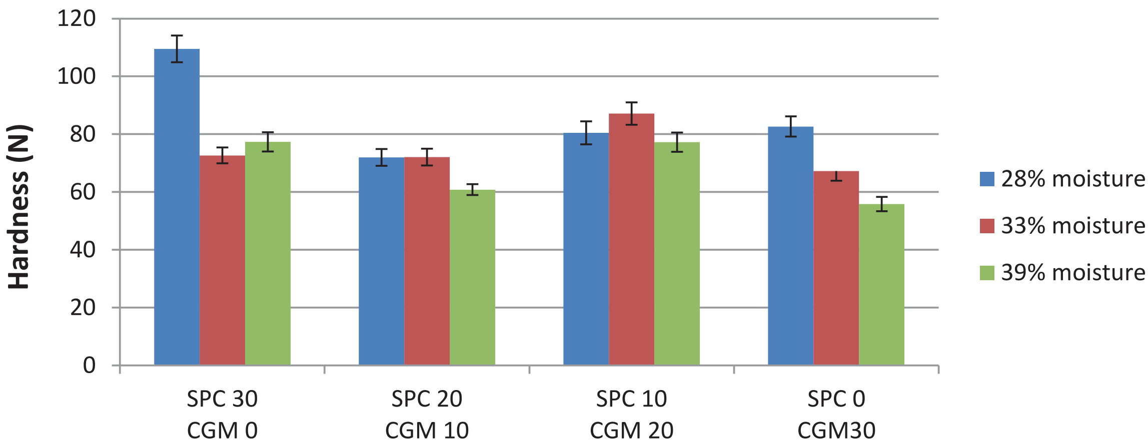

Figure 9

Hardness test – done after production. Hardness (N) of pellets is presented for different treatments. Results are presented as mean of 30 replicates ±SE

3.6 Percentage sinking pellets

Sinking percentage analyzes were performed in order to identify and compare number of sinking pellets, treated with different levels of water content, SPC and CGM (Figure 10). The reduction was observed in a diet SPC20-CGM10 treated with 28% of moisture. In addition there was no difference between other treatments where level of water content increases.

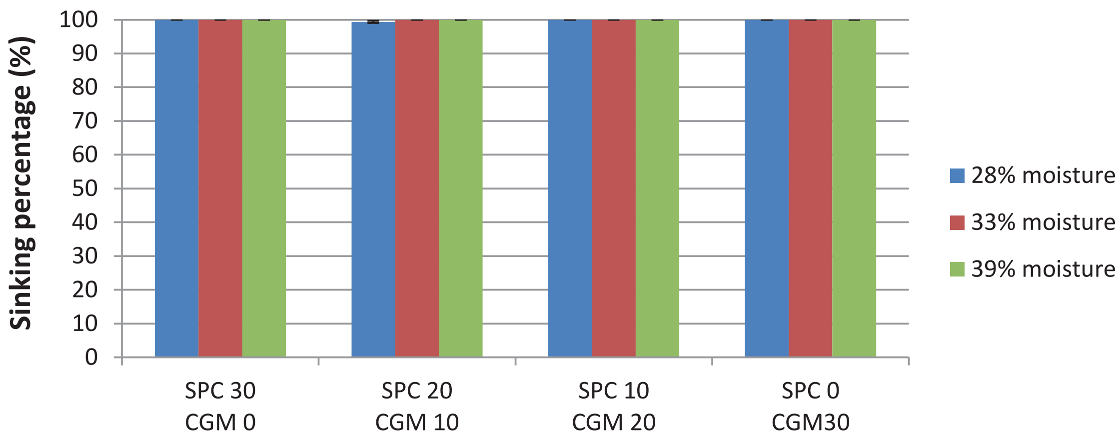

Figure 10

Sinking percentage (%) of pellets is presented for different treatments. Results are presented as mean of 3 replicates ±SE

3.7 Sinking speed

Sinking speed analyzes were performed in order to identify and compare sinking speed of pellets, treated with different levels of water content, SPC and CGM (Figure 11). In a diet SPC0-CGM30 treated with 28% of moisture, difference was observed compared to the rest of the results. Reduction in pellet sinking speed was observed in other treatments.

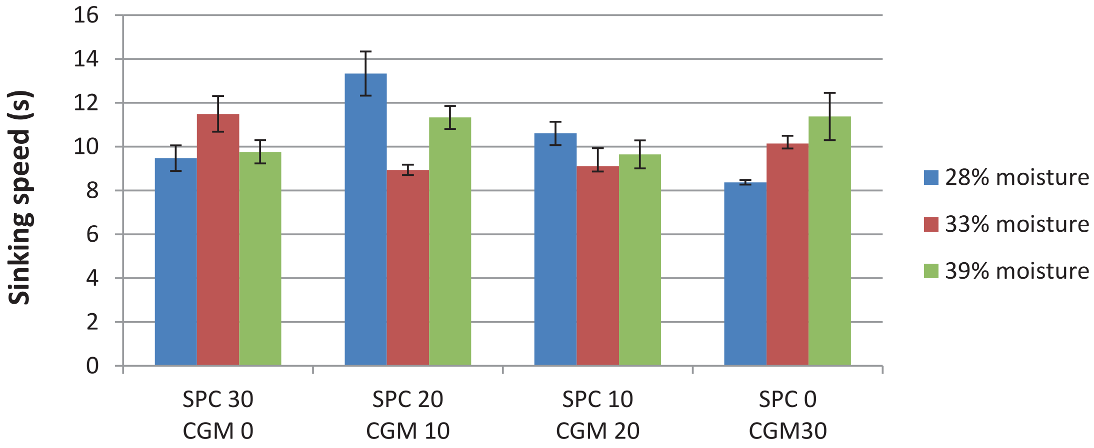

Figure 11
Sinking speed test. Sinking speed (s) of pellets is presented for different treatments. Results are presented as mean of 30 replicates ±SE

3.8 Maximal fat absorption

Maximal fat absorption analyzes were performed in order to identify and compare oil holding capacity of pellets, treated with different levels of water content, SPC and CGM (Figure 12). Differences are observed in SPC30-CGM0 and SPC20-CGM10 group compared to the rest of the results. Reduction in fat absorption was observed where the level of water content increase.

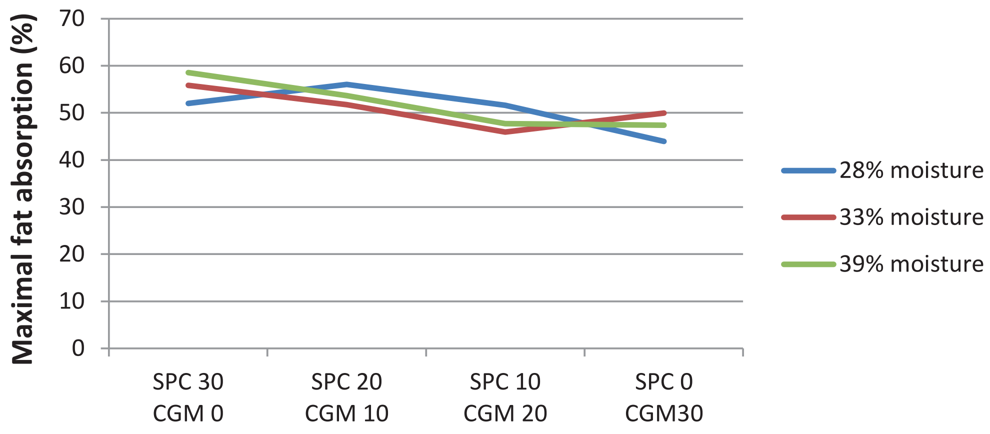

Figure 12

Maximal fat absorption. Maximal fat absorption (%) is presented for different treatments. Results are presented as mean of two replicates.

3.9 Fat leakage

Fat leakage analyzes were performed in order to identify and compare fat holding capacity of pellets, treated with different levels of water content, SPC and CGM (Figure 13). Differences were observed for SPC30-CGM0 diet treated with 33% of moisture and SPC20-CGM10 diet treated with 28% of moisture compared to the rest of the results. In addition reduction in fat leakage was observed in a diet SPC10-CGM20 treated with 33% of moisture.

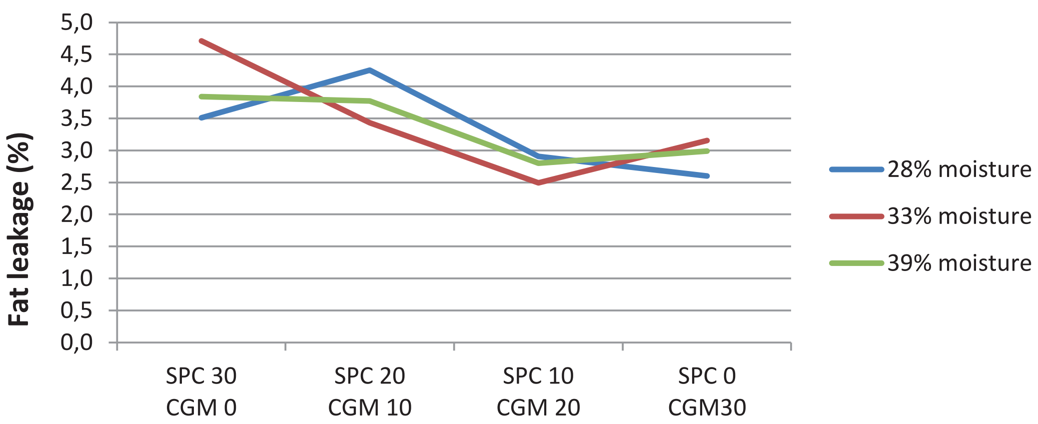

Figure 13

Fat leakage. Fat leakage (%) is presented for different treatments. Results are presented as mean of two replicates.

3.10 Specific mechanical energy

SME results were presented as readings from extruder control panel in order to compare and identify the best process economy (Figure 14). Differences were observed for SPC0-CGM30 treated with 39% of moisture and SPC30-CGM0 treated with 28% of moisture compared to the rest of the results.

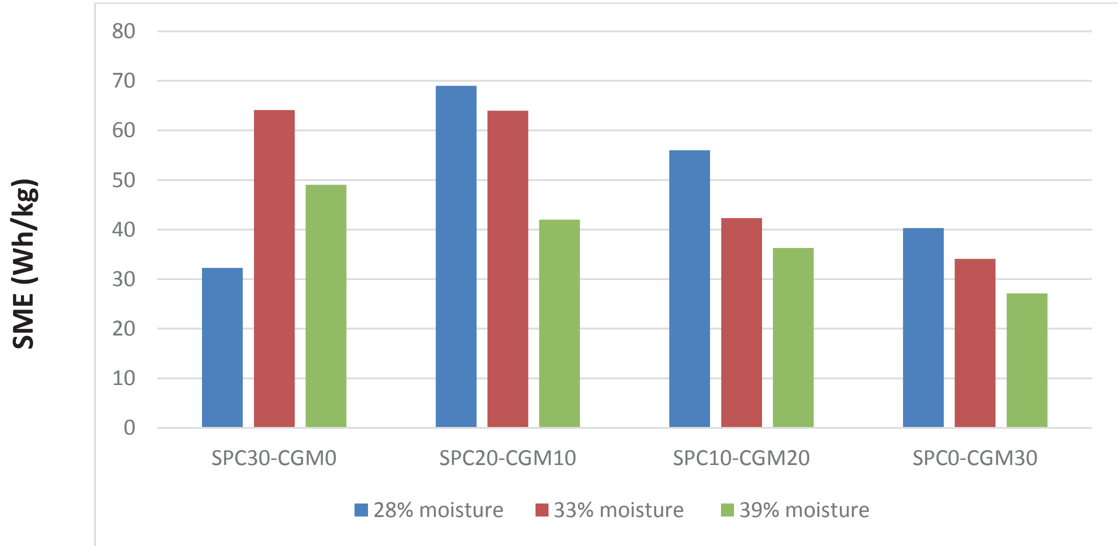

Figure 14
SME (Wh/kg of feed) is presented for different treatments.

3.11 Applied energy

Applied energy was calculated and presented as sum of SME and energy applied during the drying process in order to compare and identify the best process economy (Figure 15). Differences were observed in between the SPC30-CGM0 and SPC0-CGM30 treated with 28% of moisture, compared with the rest of the results. Differences were also observed between the same diets treated with different moisture amounts.

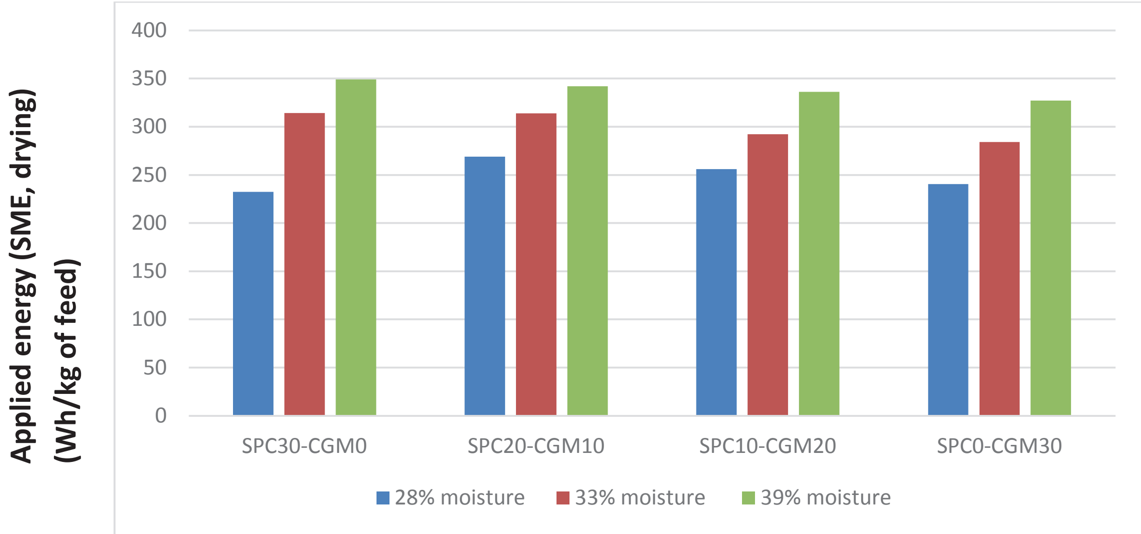

Figure 15

Applied energy (Wh/kg of feed) for different treatments.

4 Discussion

The moisture come from three sources: steam in the conditioner, water in the conditioner and water in the extruder, respectively. This was applied manually in different levels for each diet in order to reach specified bulk density. Variable feed ingredients were SPC and CGM, where SPC was gradually replaced by CGM. Generally, pellet durability and hardness are improved with an increasing amount of steam in the pre-conditioner (Thomas et.al., 1997) as well as by increasing steam pressure (Brisset, 1992). Steam addition reduces total cost (Oliveira, 1990). Due to this design, confounding with water supplementation level and the different modes of water addition, it is not possible to draw clear conclusions concerning effects of water addition or mixture rate between CGM and SPC.

The result of the experiment, however, indicated that in final product, physical properties and process economy were affected by both, replacing SPC with CGM and with an increased total amount of water. It was, however, impossible to isolate and compare the individual impact of the screw speed, conditioning temperature and water/steam addition in the extruder barrels and the conditioner regarding pellet quality. This might be explained by the large differences between and among mentioned factors.

Particle size of various ingredients is different and behave different in the grinding process (Sredanović et al., 2007). In order to achieve good homogeneity of formulated feed particles Sredanović et al., (2007) recommend that all ingredients should be grinded separately. Decrease in SME and torque was observed in CGM when the diets had higher level of particles in range from 100 to 1000 μm. This is in agreement with previous findings that particles bigger than 1000 μm affect the extrusion parameters, SME and torque (Garber et.al, 1997)

The pellet specific density is affected by many different factors such as density of ingredients, particle size distribution, amount of starch in diet, expansion rate, moisture content and fat content. Higher pellet density gives lower transportation cost (Thomas et al.,1996). Packing characteristics are changed with the different bulk density (Bühler, 2014). The pellet density was in range from 720-784 g/l (Figure 5). We observed that the diet SPC0-CGM30, treated with 28% moisture had the highest bulk density. The reason might be related to higher oil capacity. The fat leakage for the SPC-CGM30 diet was lower compared to other diets (Figure 13).

Friction between the pellets creates dust and fines with negative consequences on the environment, feed utilization and production economy (Sredanović et al., 2007). Pellet durability index (PDI) indicates amount of dust created during the transport, shearing and abrasion actions (Thomas et al., 1996). Doris durability analyzes showed similar results for both tests, after the production and six months after the storage. Decreasing durability with increasing levels of added moisture, were observed for SPC30CGM0, SPC20-CGM10 and SPC0-CGM30 diets with exception of SPC10-CGM20 diet, treated with 28% of moisture (Figures 6 and 7). Increased durability trend was found in diets with increased level of CGM (Figure 6). This confirms findings where the durability of feed was increased by replacing the crude protein from FM with crude protein of HWG (Storebakken et al., 2015b). Holmen durability analyzes showed that PDI value was in range from 99.2 to 99.7% (Figure 8.) The highest values were for diets SPC20-CGM10 treated with 33 and 39% of moisture and for a diet SPC10-CGM20 treated with 33% of moisture (Figure 8). This confirms findings of Draganović et al. (2013), where the highest durability value was related to 15% WGM diet.

The diets SPC30-CGM0 treated with 28% of moisture, SPC10-CGM20 treated with 33% of moisture and SPC0-CGM30 treated with 28% of moisture, showed best result for hardness higher than 80N (Figure 9). Lower hardness in other treatments was observed. No obvious trend was observed by replacing the SPC with CGM neither with differences in total water amount.

Sinking percentage values were 100% for all diets with an exception in diet SPC20-CGM10 treated with 28% of moisture where the sinking percentage was 99.3% (Figure 10). The reasons might be low pellet density (Figure 5), high fat leakage (Figure 13) and low sinking speed (Figure 11) for this specific diet. Sinking speed was in range from 8.2 to 13.2 m/s (Figure 11). No obvious trend was found by replacing the SPC with CGM neither with differences in total water amount. Storebakken et al., (2015b) had the similar results by replacing FM with HWG.

Lipids are the main energy source in salmonids. High oil holding capacity of pellets is required in a fish feed. The higher oil absorption was found in SPC20-CGM10 diet treated with 28% of moisture and SPC30-CGM0 diet treated with 33% of moisture (Figure 12). This confirms the findings of Draganović et al., (2013) where the feed with up to 15% of wheat gluten meal (WGM) had greater oil absorption. Draganović et al., (2013) also found reduction in oil infusion when the level of WGM level was higher than 15% in a diet, what confirms our linear reduction for diets SPC30-CGM0, SPC10-CGM20 and SPC10-CGM20 treated with 33 and 39% (Figure 12). Maximal fat absorption was in range from 46 to 59%. Targeted oil capacity was 34.5% so we can conclude that all diets satisfy required projection. Diet SPC30-CGM0 treated with 39% of moisture followed with diets SPC20-CGM10 treated with 28% of moisture and SPC30-CGM0 treated with 33% of moisture had the highest fat leakage. The same diets had the highest maximal fat absorption (Figure12). Diets SPC10-CGM0 treated with 33% of moisture and SPC0-CGM30 treated with 28% of moisture, had the lowest fat leakage. Fat leakage test showed similar trend to the findings of Draganović et al., (2013), where the fat leakage decreased with by increasing WGM from 5-20%.

Dry feed, with moisture less than 10% is used for salmonids. Extrusion, conditioning and drying participate with 72% of feed processing energy cost where 21% is of cost is used for conditioning and pellet forming and 51 for drying (Skretting, 2013). SME readings showed higher SME with increased moisture for all diets (Figure 14). Reduction in SME was found in diets where CGM content increases from 10 to 30%. The best result was 27.1 Wh/kg, found in a diet SPC0-CGM30 treated with 39% moisture. The reasons might be better flowing properties of CGM compared with SPC (Figure 4), low screw speed (Table 2) and high moisture content (Table 3). These results partially match Storebakken et al. (2015b) findings in an experiment with hydrolyzed wheat gluten (HWG), where the SME was decreased with increased level of crude protein from HWG. In the same experiment SME increased when the crude protein content of HWG exceeded 25%. Our result confirms findings of Bordoloi & Ganguly (2014) that the SME decreased by the increased moisture content during the extrusion of maize grits. Draganović et al., (2013), in the experiment with WGM had the similar result where the SME had decreasing trend with increased level of WGM. Zhu et al., (2010) reported that expansion of extrudate decrease when SPC increase from 20-30% in a diet with corn starch. Applied energy for all diets was affected by increasing moisture content. Reduction in applied energy was found in diets where CGM content increases from 10 to 30%. The lower energy cost was 227.1 Wh/kg, found in a diet SPC0-CGM30 treated with 28% moisture.

5 Conclusion

This experiment was driven by an aim to achieve specified bulk density of extruded pellets, identify production parameters, with optimal physical pellet quality and lowest production costs. Specific effects of water addition or ratio between CGM and SPC may have been masked by different procedures applied for water addition within each combination of protein source and water supplementation level. Based on the results obtained in this study it can, however, be indicated that CGM has the potential to replace SPC in extruded feeds without negative effects on physical quality or process economy.

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